to O1, O2, and O3 operators in the *lac* operon. Specifically, we demonstrated that LacI kept two supercoils within the 401 bp DNA-loop between *O1* and *O2* operators. Additionally, We carried out time course studies to determine the stability of the topological barriers that are produced by the different LacI-operator complexes. Our results showed that the stability of the topological barriers correlates with the DNA-binding affinity of LacI to the different operators i.e., *O1*, *O2*, *O3*, and *Os* operators. Furthermore, we confirmed our previous observation in which LacI is able to ''keep'' certain superhelical energy to stabilize LacI-*lacO1* complexes. Our results can be explained by a model in which LacI behaves as a topological barrier in the *lac* operon to regulate the expression of *lacZYA* genes in *Escherichia coli* cells.

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Flanking DNA Matters in DNA Loop Formation and Breakdown Mediated by Lac Repressor Protein

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The lac Repressor protein (LacI) is a paradigm for the study of protein-mediated DNA loops in bacteria. When it binds to two distant operator sites on substrate DNA, it causes the formation of DNA loops. Although past in vitro and in vivo experiments have shown that changes in the inter-loop DNA can affect loop formation and breakdown, here I will present in vitro experimental evidence that the DNA outside the loop that is flanking the operators can also affect looping kinetics. Observing loop formation and breakdown in single molecules with Tethered Particle Motion (TPM) we found fluctuations in how much DNA is bound to the protein, and these fluctuations depend on the AT- or GC-content of the flanking regions outside the loop. This suggests an interaction between the looped complex and the flanking region which has not been previously observed. The studies complement previous experiments which found that DNA between the operator sites interacts with LacI while in the looped state. These results suggest that the presence or absence of flanking DNA interactions with the protein complex could give rise to additional states in TPM experiments that are not caused by conformational changes of the protein complex or topologic variations of the loop.

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DNA Competition Experiments Reveal the Importance of Operator Binding Strength and Inter-Operator Sequence in Protein-Mediated DNA Looping

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DNA looping mediated by the lac Repressor protein (LacI) is a paradigm of study for protein-DNA interaction as well as DNA mechanics on the nanoscale. Loop formation in this system occurs by the spontaneous binding of LacI to two distant operators on its DNA substrate. We employ single-molecule Tethered Particle Motion (TPM) to observe loop formation and breakdown in various DNA substrates. We are discussing and aiming to explain substantial differences between loop lifetime measurements in bulk and single-molecule experiments, namely an unexpected difficulty in competing bound protein off in the presence of excess DNA even though loops continued to form and break down rapidly. For this aim, we conduct single-molecule DNA competition experiments using DNAs with different operator strengths and intra-operator sequences giving rise to intrinsic bends. We find that we are able to compete off LacI that is bound to unbent DNA constructs with non-ideal operators in a matter of minutes, in line with typical loop breakdown rates. In contrast, competing off LacI bound to unbent or intrinsically curved DNA substrates with ideal operators took at least hours up to days in both single-molecule and bulk experiments, even though loops continue to break down and form repeatedly within minutes. To explain this resistance to competition of the LacI-DNA complex in DNA substrates with ideal operators while loops continue to break down, we posit a weak binding of LacI to non-operator DNA in the unlooped state; this hypothesis is supported by recent complementary experiments that reveal interactions between LacI and non-operator DNA outside the looping region.

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Analysis of DNA Looping Kinetics in Tethered Particle Motion Experiments using Hidden Markov Models

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Tethered particle motion (TPM) is a powerful method for measuring DNA-protein interactions at the single molecule level. TPM experiments monitor the Brownian motion of beads tethered to a microscope cover slip. The Brownian motion changes when a protein binds to and deforms the DNA 'leash', for example in the formation of a DNA loop. A complicating factor in the interpretation of TPM data is that the number of observable states, corresponding to different conformations of the DNA-protein complex, is often not known in advance. Moreover, conformational transitions that occur on time scales comparable to the diffusive motion of the bead are difficult to extract from the data. We present an analysis method for TPM data that overcomes these limitations in existing approaches. Our method relies on variational Bayesian inference on a variant of the Hidden Markov model. This variational approach allows us to determine the number of states directly from the data in a statistically principled manner.
Moreover, by operating directly on the position data, we achieve significantly better time resolution compared to methods based on running averages of the bead root-mean-square distance from the tethering point. Finally, we show that hierarchical techniques developed in the context of single molecule FRET experiments can be adapted to our TPM methods to perform pooled analysis on many trajectories. This increases the accuracy of the method despite considerable bead-to-bead variability, and allows a more precise characterization of rare events.
We apply our method to Lac-mediated loop formation on a short (107 bp) construct, and demonstrate direct interconversion between two different looped states, with implications for structual models of the looped Lac-DNA complex.

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Binding to Different Homologous DNA Sequences by a Protein alters the Dynamical Properties of the Bound Protein in a DNA Sequence Dependent Manner: Operator DNA Induced Allostery in the Structure and Dynamics of Lambda Repressor

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Δ-repressor-operator sites interaction, particularly O_R1 and O_R2, is a key component of the Δ-genetic switch. The N-terminal domain of the protein Δ-repressor interacts with the DNA, while the C-terminal domain is responsible for most of the protein–protein interactions, essential for the co-operative binding and for the functioning of the genetic switch. Frster resonance energy transfer from the dansyl, bound to the C-terminal domain of the protein, to the intercalated EtBr in the operator DNA, indicates that the structure of the protein is more compact in the O_R2 complex than in the O_R1 complex. We have explored the photoinduced electron transfer process from the tryptophan moieties of Δ-repressor to O_R1 and O_R2 DNA to verify the conformational differences of the C-terminal domain of the repressor, bound to O_R1 and O_R2 DNA. Most importantly, fluorescence anisotropy study reveals enhanced flexibility of the C-terminal domain of the repressor at ultrafast timescales upon complexation with O_R1. In contrast, O_R2 bound repressor shows no significant enhancement of protein dynamics at these timescales. Moreover, sedimentation equilibrium study reveals that this differential dynamics is important for correct protein-protein interactions between two Δ-repressor dimers bound to O_R1 and O_R2, for the functioning of the genetic switch. Hence, we demonstrate that binding of transcription factors to specific DNA sequences alters the dynamical properties of the bound protein in a DNA-sequence dependent manner and the dynamical difference contributes to the formation of correct regulatory complex.
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The Different FMRP Isoforms Bind with High Affinity to the G-Quadruplex formed by the FMRP mRNA

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Fragile X syndrome, the most common form of inherited mental retardation in humans, affects about 1 in 3000 males and 1 in 5000 females. It is caused by the loss of expression of the fragile X mental retardation protein (FMRP) due to a CGG trinucleotide repeat expansion in the 5'-untranslated region (UTR) of the fragile x mental retardation-1 (fmr1) gene. FMRP has been shown to use its arginine-glycine-glycine (RGG) box RNA binding domain to bind with high affinity and specificity to G quadruplex forming mRNA sequences. The binding of FMRP to a proposed G quadruplex structure in the coding region of its own mRNA (100 nucleotide fragment named FBS) has been proposed to affect mRNA splicing events for isoforms 1 through 3. In this study we truncated the original 100 nt FMRP-FBS to 42 nt and used biophysical methods to directly demonstrate its folding into a G-quadruplex structure and the binding affinity of the different FMRP isoforms to it.